IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent Application No.: 10/535,500

Filed: May 26, 2006

Attorney Docket No.: 55320.001041

Confirmation No. 7327

Art Unit: 1643

Examiner: Gussow, Anne.

Title: Methods and kits for diagnosing and treating B-Cell Chronic Lymphocytic Leukemia (B-CLL)

(SECOND) § 1.132 AFFIDAVIT BY THE INVENTORS

We, Anne Mette Buhl Hertz and Henrik Leffers declare and state as follows:

(1) That we are two of the inventors of the above-identified application.

(2) That in that capacity we jointly prepared and executed an invention disclosure relating to the above-identified patent application. We have assigned all of our right, title and interest to the invention to the proprietor, CLLUOne Diagnostics A/S, a company of which we are part owners.

(3) That we reviewed the Office Action dated July 15, 2008 in the above-identified patent application relating to methods and kits for diagnosing and treating B-Cell Chronic Lymphocytic Leukemia (B-CLL).

(4) That based thereon we understand that the Examiner has concluded that the claims are lacking enablement. While we are not legal experts we understand from our ordinary reading of the Office Action and the rejection of our invention is that the Examiner has concerns as to the efficacy of the claimed methods of disease detection which rely on the detection of the presence or absence of specific expression products.

(5) That we respectfully disagree with the rejection as we both are of the opinion, based on our expertise in the relevant art, that the as-filed application contains sufficient information for one skilled in the art to practice the claimed methods which are directed to detecting a specific subtype of B-cell chronic lymphocytic chronic lymphocytic leukemia (B-CLL) in an individual (which subtype is correlated with poor disease prognosis) by detecting the presence or absence of at least one expression product, wherein said at least one expression product comprises a nucleotide sequence selected from the group consisting of SEQ ID No: 13, SEQ ID No: 15, SEQ ID No: 16, or SEQ ID No: 17 in a biological sample isolated from the individual. However, in order to further demonstrate the efficacy and reliability of the claimed methods of detecting a particularly virulent form of B-CLL the declarants provide herewith supplementary data including Northern data supporting the efficacy of the claimed invention. The declarants note that this Northern data was generated prior to the filing of PCT application PCT/DK03/00794 which provides the basis of this US patent application and which PCT was filed on November 19, 2003. This supplementary data and the information contained in the as-filed application are representative data of a significant amount of experimental data, which are consistent in that they support the efficacy of claimed invention. This

supplementary data is attached as Exhibit A and B to this Declaration and is summarized below.

(6) The newly provided Northern data (Exhibit A) are two phosphor imager images which are from the same Northern blot probed with two different probes, an Exon-3 and an Exon-1 probe. The blot which was probed with Exon-3 has been inverted (mirrored) to facilitate comparison of the hybridizations.

Aliquots of RNAs isolated from white blood cells of B-CLL patients were loaded on an agarose gel, which were subjected to electrophoresis. The agarose gel was blotted to a filter: Lane 1-5 (referring to the numbers on the images on the left, Exon-1 probing), 5 RNA samples from 5 patients with CLL and un-mutated Ig genes (poor prognosis); lane 6-8, RNA from 3 patients with CLL and mutated Ig (good prognosis).

The filter (i.e. the Northern blot) was first hybridized with a probe corresponding to Exon-3 (SEQ ID NO: 16). The probe was fully included in Exon-3. After exposure to phosphor imaging the filter was stripped from the Exon-3 probe by boiling and subsequently hybridized with a probe corresponding to Exon-1 (SEQ ID NO: 16), the probe includes almost all of Exon-1 and nothing else. The two probes generate identical hybridization patterns. The identity of the bands is indicated: at the top is a band corresponding to the primary transcript, which starts with Exon-1 and ends at the end of Exon-3 without splicing. The two bands correspond to the two versions of the most abundant spliced transcript (Exon-1- Exon-3); the short version utilizes an alternative poly-adenylation site in Exon-3, upstream from the one used in the long form, resulting in a shorter transcript.

(7) In addition in Exhibit B we investigated the relative expression levels of the main CLLU1 transcripts by quantitative PCR (QRT-PCR), using primers that amplified across exon splice sites (for transcripts cDNA1/1a and cDNA1/4) or internal in the putative coding region (CDS). The position of the primers is shown in Fig. 1. The comparisons were made by running QRT-PCR on many patients with the three primer pairs and then compare their relative expression levels (Figs 2 & 3). For all transcripts we found a linear correlation between the expression levels of the different transcripts, i.e. the relative patient-dependent expression level of CLLU1 was independent of which transcript we quantified. This can only be explained by the presence of a primary transcript that is spliced (or un-spliced) to the detected mRNAs, which are present in different relative amounts depending on the efficiency of the individual splicing reactions. This is determined by the sequences flanking the splice sites and therefore patient independent. Thus, the transcript corresponding to cDNA1 (exon1-exon3) is the most efficiently spliced transcript and is therefore present in largest amount in all the patients and therefore detectable with lower ΔCt numbers than the less efficiently produced (spliced) transcript corresponding to cDNA2/4 in Fig. 2. Since the ΔCt values were normalized to the levels in B-cells in Fig. 3, the two transcripts (cDNA1 and CDS) appear with equal values because the splicing efficiency (i.e. splicing of exon1-3 and lack of splicing for CDS) of the two transcripts as expected is conserved between CLL- and B-cells. However, the actual level of cDNA1 is in all samples in fact much higher than the actual level of mRNA containing the CDS (as seen in Fig. 2 for cDNA1 and cDNA2/4).

(8) That based on this newly provided data as well as the information contained in the as-filed application the undersigned respectfully submit that the rejection under 35 U.S.C. 112, first paragraph as lacking enablement should be withdrawn since the newly submitted supplementary data together with information in the as-filed application clearly establishes that the claimed methods which are directed to detecting a specific subtype of B-CLL in an individual by detecting the presence or absence of at least one expression product in a biological sample isolated from said individual, (wherein said at least one expression product comprises a nucleotide sequence selected from the group consisting of SEQ ID No: 13, SEQ ID No: 15, SEQ ID No: 16 or SEQ ID No: 17) are effective and could be practiced by one of ordinary skill in the art using the information and methods disclosed in the as-filed application.

(9) All statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like may jeopardize the validity of the application or any patent issuing thereon.

14/01-2009
Date

Anne Mette Buhl Hertz

Henrik Leffers

EXHIBIT A

Exon 1 Northern

Exon 3 Northern

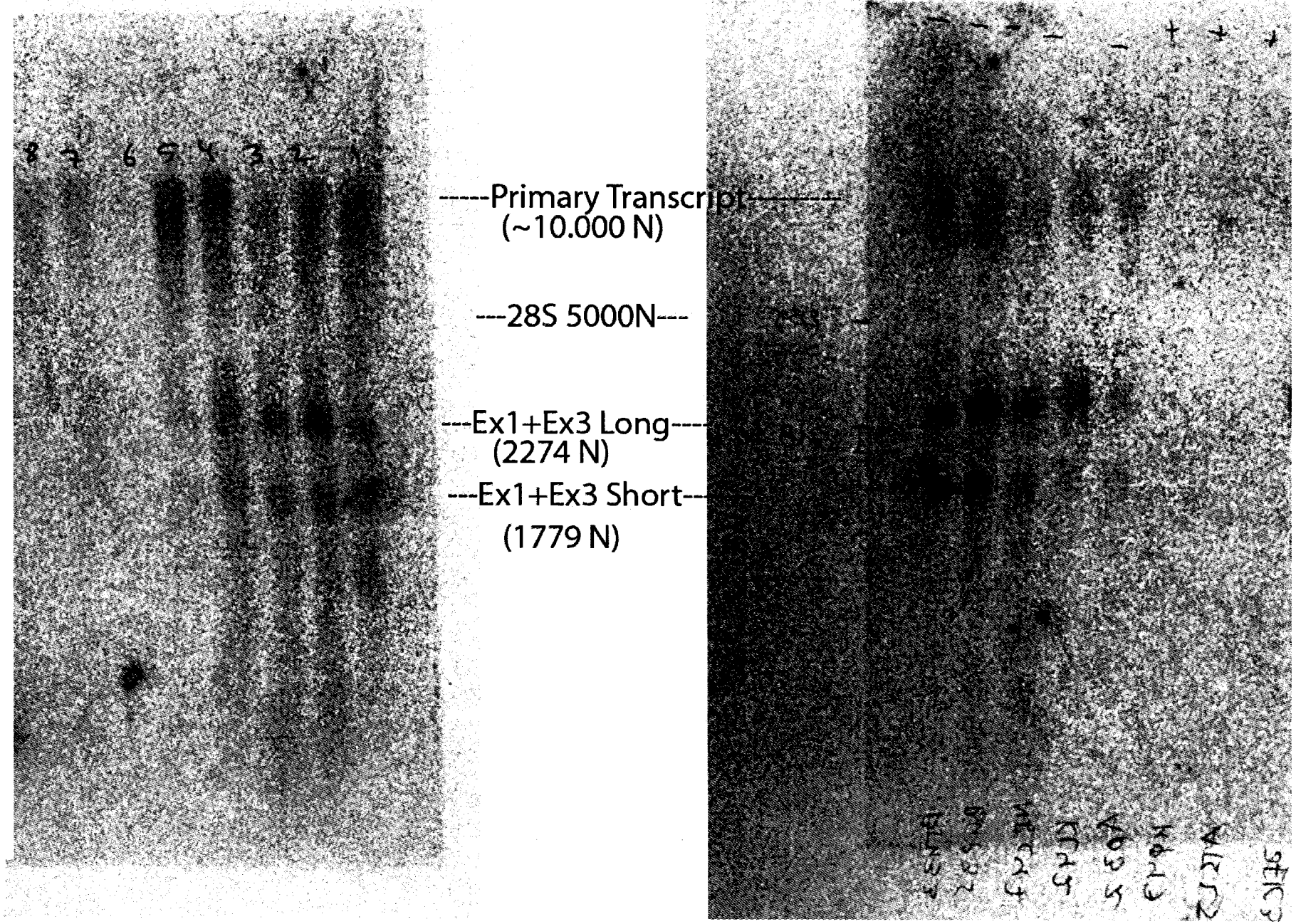

EXHIBIT B

PCR analysis show correlated expression of all CLLU1 transcripts

We investigated the relative expression levels of the main CLLU1 transcripts by quantitative PCR (QRT-PCR), using primers that amplified across exon splice sites (for transcripts cDNA1/1a and cDNA1/4) or internal in the putative coding region (CDS). The position of the primers is shown in Fig. 1.

The comparisons were made by running QRT-PCR on many patients with the three primer pairs and then compare their relative expression levels (Figs 2 & 3). For all transcripts we found a linear correlation between the expression levels of the different transcripts, i.e. the relative patient-dependent expression level of CLLU1 was independent of which transcript we quantified.

This can only be explained by the presence of a primary transcript that is spliced (or un-spliced) to the detected mRNAs, which are present in different relative amounts depending on the efficiency of the individual splicing reactions. This is determined by the sequences flanking the splice sites and therefore patient independent. Thus, the transcript corresponding to cDNA1 (exon1-exon3) is the most efficiently spliced transcript and therefore present in largest amount in all the patients and therefore detectable with lower ΔCt numbers than the less efficiently produced (spliced) transcript corresponding to cDNA2/4 in Fig. 2. Since the ΔCt values were normalised to the levels in B-cells in Fig. 3, the two transcripts (cDNA1 and CDS) appear with equal values because the splicing efficiency (i.e. splicing of exon1-3 and lack of splicing for CDS) of the two transcripts as expected is conserved between CLL- and B-cells. However, the actual level of cDNA1 is in all samples in fact much higher than the actual level of mRNA containing the CDS (as seen in Fig. 2 for cDNA1 and cDNA2/4).

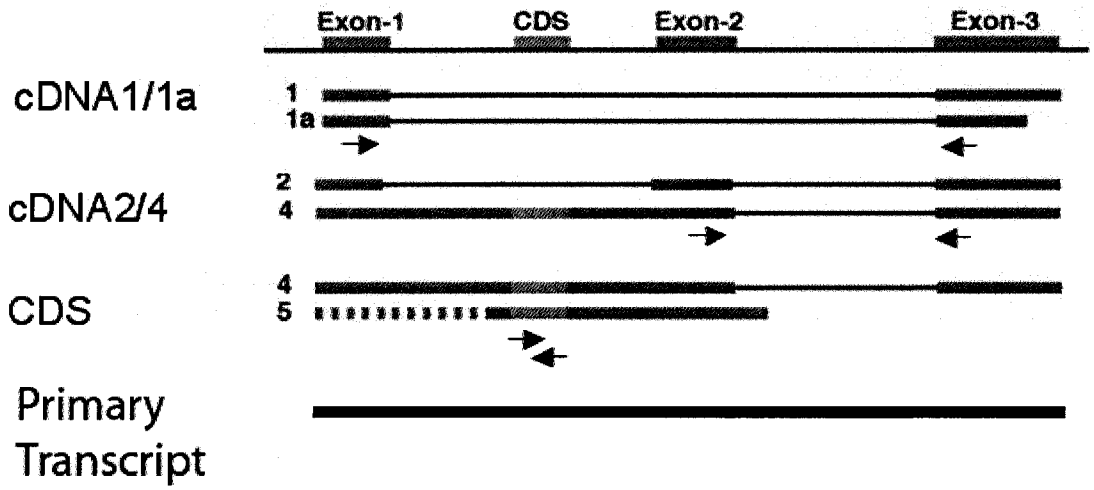

Fig. 1. Overview of the region of chromosome 12q22 where CLLU1 is located. The position of the primers that were used to quantify the expression levels of transcripts cDNA1/1a, cDNA2/4 and the putative coding region (CDS) in QRT-PCR are shown.

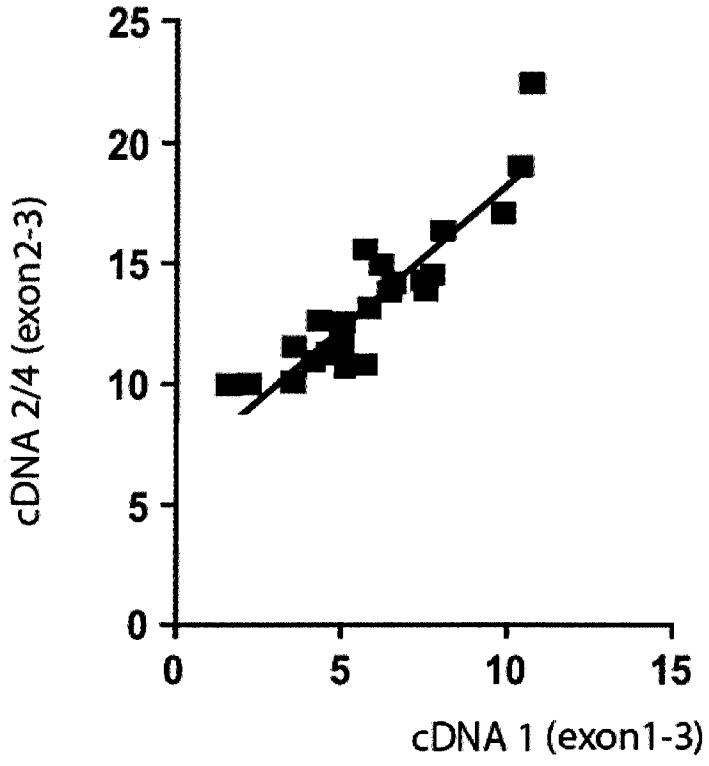

Fig. 2. Comparison of cDNA1 and cDNA2/4 expression levels in a range of CLL patients. X-axis: cDNA1 Y-axis :cDNA2/4, r=0.90, p<0.0001. The axes show ΔCt values, which are the number of PCR cycles required to reach the threshold level for CLLU1 after normalization to the β2-Microglobulin internal standard (adapted from Buhl et al., 2009).

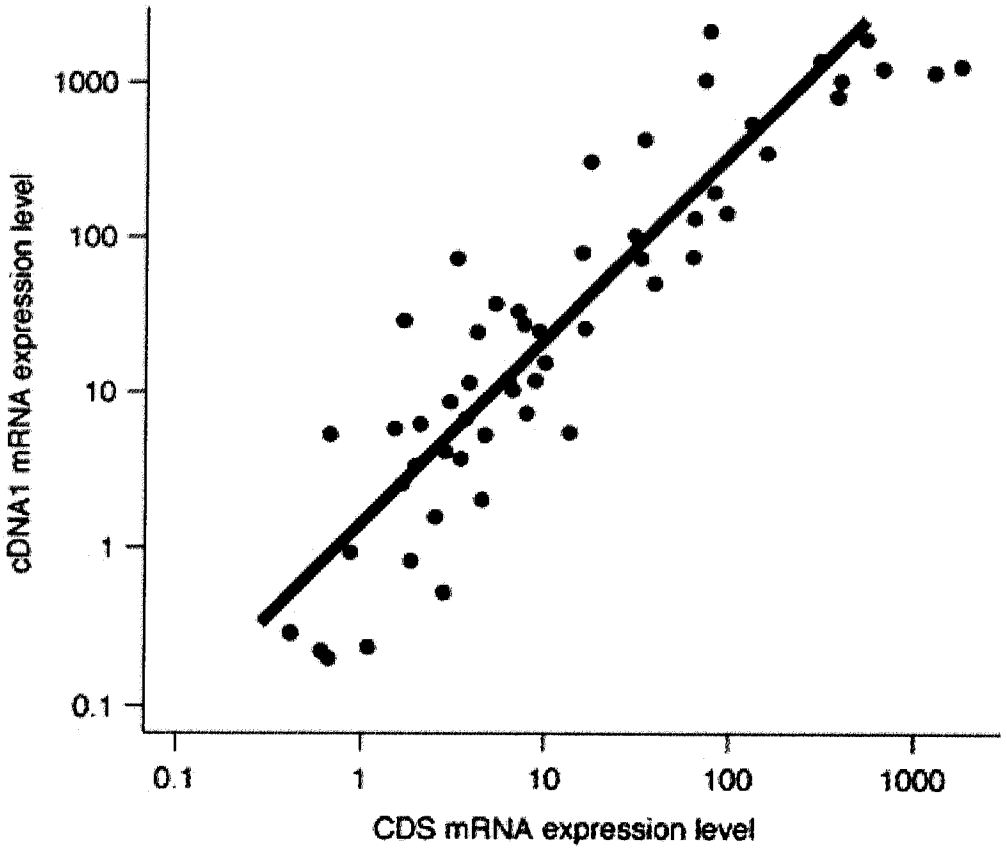

Fig. 3. Comparison of cDNA1 and CDS expression levels in a range of CLL patients. Each dot represents a CLL patient from our study material and the patients that were analysed for cDNA1–cDNA2/4 expression in Fig. 2 were also included in this analysis, together with additional patients. The ΔCt values were first determined by normalization to the β2-Microglobulin internal standard (as in Fig. 2) and then divided with the ΔCt values for B-cells. Thus, the axes show fold expression as compared with the expression in normal B-cells (adapted from Buhl et al., 2006).

References:

Buhl AM, Jurlander J, Geisler CH, Pedersen LB, Andersen MK, Josefsson P, Petersen JH, Leffers H (2006). CLLU1 Expression Levels Predict Time to Initiation of Therapy and Overall Survival in Chronic Lymphocytic Leukemia. Eur. J. Haematol. 76, 455-464

Buhl AM, Novotny GW, Josefsson P, Nielsen JE, Pedersen LB, Geisler C, Rassenti LZ, Kipps TJ, Jurlander J, Leffers H (2009). The CLLU1 expression level is a stable and inherent feature of the Chronic Lymphocytic Leukemia clone. Leukaemia, accepted for publication.